β-Arrestin Goes Nuclear

Jean-Martin Beaulieu[1] and Marc G. Caron[1,*]
[1]Departments of Cell Biology and Medicine, Duke University Medical Center, Durham, NC 27710, USA
*Contact: m.caron@cellbio.duke.edu
DOI: 10.1016/j.cell.2005.11.010

Arrestins have important roles in the cytoplasm and at the plasma membrane, including the desensitization and internalization of G protein-coupled receptors (GPCRs). In this issue of *Cell*, Kang et al. (2005) provide evidence that β-arrestin 1 moves to the nucleus in response to GPCR stimulation, where it regulates gene expression by facilitating histone acetylation at specific gene promoters.

Arrestins are a family of scaffolding proteins that are closely associated with the function of seven transmembrane domain G protein-coupled receptors (GPCRs). In mammals, two members of the arrestin family, the visual arrestins, are restricted to photoreceptor cells, whereas two others, β-arrestin 1 and β-arrestin 2, are expressed ubiquitously. These proteins promote the internalization and desensitization of GPCRs (Ferguson et al., 1996; Lohse et al., 1990). Following agonist-induced phosphorylation of GPCRs by kinases, arrestins move from the cytoplasm to the plasma membrane and bind to the phosphorylated receptors. The interaction of arrestins with phosphorylated GPCRs leads to an uncoupling of G protein-dependent receptor signaling (receptor desensitization). Arrestins also promote receptor internalization by recruiting the endocytic machinery. However, recently it has become evident that the biological functions of arrestins go well beyond these well-established roles.

It is becoming clear that arrestins promote signal transduction by GPCRs. Both β-arrestin 1 and β-arrestin 2 act as molecular scaffolds that recruit signaling molecules such as kinases and phosphatases to the activated receptors (Beaulieu et al., 2005; Luttrell et al., 1999; Shenoy and Lefkowitz, 2003). Formation of β-arrestin-based signaling complexes facilitates the activation and/or inhibition of these signaling molecules (Shenoy and Lefkowitz, 2003). The scaffolding/signaling functions of β-arrestins have been studied extensively in heterologous and homologous cellular systems, and in vivo a β-arrestin 2/PP2A/Akt signaling complex is critical for D2 dopamine receptor-dependent behaviors in mice (Beaulieu et al., 2005).

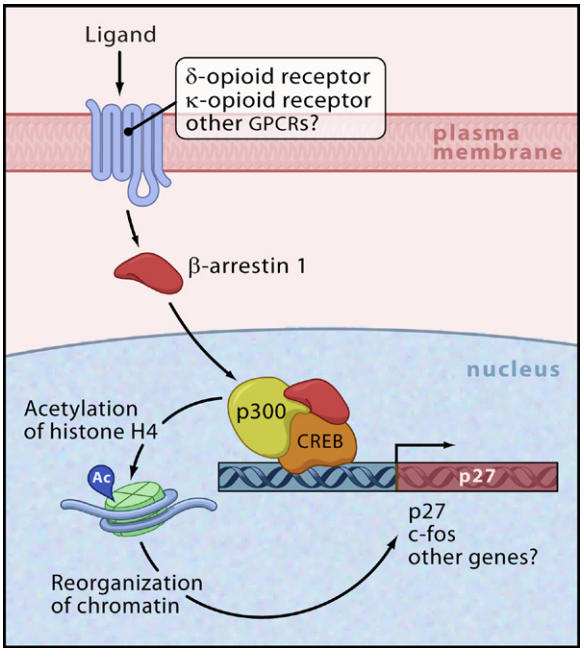

Figure 1. Model for the Regulation of Histone Acetylation and Gene Transcription by β-Arrestin in Response to GPCR Activation

Stimulation of δ- and κ-opioid receptors and potentially other GPCRs results in enhanced translocation of β-arrestin 1 to the nucleus and its association with specific promoter regions such as those for the *c-fos* and *p27* genes. β-arrestin 1 in the nucleus acts as a scaffold for a protein complex, which includes the CREB transcription factor, the histone acetyltransferase p300, and other unidentified proteins. Recruitment of p300 to specific promoter regions by β-arrestin 1 results in acetylation (Ac) of histone H4 leading to chromatin reorganization and enhanced gene transcription.

During development, β-arrestins may have a role in signaling by the seven transmembrane domain receptor proteins frizzled and smoothened (Chen et al., 2004). β-arrestins translocate to these receptors in a phosphorylation-dependent manner. In zebrafish, downregulation of β-arrestin 2 results in a disruption of hedgehog/patched/smoothened function during development (Wilbanks et al., 2004), suggesting that β-arrestins act either as positive regulators or as mediators of signaling in these systems.

In this issue of *Cell*, Kang et al. (2005) have uncovered yet another unexpected function of arrestins. Since their initial characterization, arrestins have been thought of as cytoplasmic proteins that could be recruited to the plasma membrane and endocytic compartments following receptor activation. Although β-arrestin 2 and visual arrestins appear to be found exclusively in

the cytoplasm, β-arrestin 1 has been shown to reside in both the cytoplasm and the nucleus (Scott et al., 2002; Wang et al., 2003), thus suggesting that at least one member of this family may also have a nuclear function. Intrigued by this possibility, Kang et al. (2005) assessed the impact of GPCR stimulation on β-arrestin 1 distribution and showed that in transfected fibroblasts, β-arrestin 1 translocates to the nucleus in response to activation of two prototypical GPCRs, the κ- and δ-opioid receptors. Furthermore, translocation of β-arrestin 1 to the nucleus correlated with an increase in the expression of two genes involved in the regulation of cell proliferation, the transcription factor *c-fos* and the cyclin-dependent kinase inhibitor *p27/kip1*. Activation of κ-opioid receptors also leads to an increase in the level of p27 protein that is dependent on nuclear β-arrestin 1. A thorough characterization of the molecular events by which β-arrestin 1 contributes to the regulation of these two genes revealed that β-arrestin 1 participates in the formation of a nuclear complex on the promoter regions of the *p27* and *c-fos* genes. This complex includes the transcription factor CREB and the histone acetyltransferase p300. β-arrestin 1 does not appear to be essential for the binding of CREB to *c-fos* and *p27* gene promoters. From these data, the authors suggest that β-arrestin 1 acts as a nuclear scaffold that recruits p300 to CREB. This leads to increased acetylation of histone H4 and the reorganization of chromatin, thereby increasing gene expression (Figure 1).

The results of Kang et al. (2005) are potentially groundbreaking because they introduce a completely new function for arrestins and a new mode by which GPCRs may regulate gene transcription. GPCR signaling through traditional G protein-dependent and second messenger cascades have previously been shown to regulate gene expression, and these pathways presumably regulate histone modification and chromatin remodeling (Chao and Nestler, 2004). However, the involvement of β-arrestin in mediating GPCR regulation of transcription may be required in certain physiological situations. An interesting distinction that has become apparent from cellular and in vivo studies is that β-arrestin-dependent GPCR signaling lasts longer than conventional G protein-dependent signaling (Ahn et al., 2004; Beaulieu et al., 2005). Thus, the β-arrestin-dependent mechanism for transcriptional control as proposed by Kang et al. (2005), may be used under certain physiological situations when sustained signaling is needed.

As with any provocative new findings, the model proposed by Kang et al. (2005) raises multiple questions that may fuel research for years to come. A major question concerns to what extent this mechanism is used by GPCRs. The authors have limited their observations to a few GPCRs. δ- and κ-opioid receptors trigger nuclear accumulation of β-arrestin 1, whereas stimulation of μ-opioid and β-adrenergic receptors do not. However, it is highly possible that other GPCRs and perhaps non-GPCR receptors may also trigger translocation of β-arrestin 1 to the nucleus. In this regard, it is particularly intriguing from the results presented by Kang et al. (2005) that both *c-fos* and *p27* gene expression were affected by changes in the expression of β-arrestin 1 whereas only *p27* expression was clearly affected by δ-opioid receptor stimulation. This suggests that other receptor systems participate in the regulation of *c-fos* expression by β-arrestin 1. Similarly, it is an open and rather obvious question whether *c-fos* and *p27* are the only two genes that can be regulated by β-arrestin 1. One would think that β-arrestin 1 mediates widespread changes in gene expression.

Another question concerns the mechanisms by which β-arrestin 1 nuclear complexes are formed. Kang et al. (2005) state that a preliminary proteomic characterization of the complex indicates the presence of other proteins in addition to β-arrestin 1, CREB, and p300. Characterization of these proteins may reveal other direct interacting partners of β-arrestin 1 and could suggest a mechanism by which GPCRs regulate β-arrestin 1 translocation to the nucleus and gene expression. Another particularly interesting aspect of the mechanism proposed by Kang et al. (2005) is that receptors may not be part of the nuclear complex they describe, thus raising the tantalizing possibility that arrestins may act as molecular scaffolds in the absence of receptors. β-arrestins have been shown to act as scaffolds for multiple signaling kinases, some of which, like Erk and Akt, are known to undergo nuclear translocation (Beaulieu et al., 2005; Luttrell et al., 1999). Thus, it would be interesting to determine whether nuclear β-arrestin 1 can act as a scaffold for the phosphorylation of nuclear proteins in addition to modulating histone acetylation.

Finally, as with any work conducted in heterologous cellular systems, it is uncertain under which conditions the new nuclear function of β-arrestin 1 will be physiologically relevant. Although the authors make a valiant effort to evaluate the impact of δ-opioid receptor stimulation upon p27 expression and histone H4 acetylation at the *p27* promoter in the brain, more detailed studies will be required to clearly establish the role of β-arrestin 1 in these processes in vivo. Moreover, the absence of an overt developmental phenotype in β-arrestin 1 knockout mice suggests that the role played by this protein in gene regulation under physiological conditions may be limited or compensated by other mechanisms. However, a detailed reexamination of β-arrestin 1 knockout mice in light of the observations made by Kang et al. (2005) may reveal subtle phenotypes and allow the identification of as-yet-unrecognized biological functions of β-arrestin 1.

ACKNOWLEDGMENTS

Authors are supported by NARSAD (M.G.C.; J.-M.B.) and CIHR (J.-M.B.).

REFERENCES

Ahn, S., Shenoy, S.K., Wei, H., and Lefkowitz, R.J. (2004). J. Biol. Chem. *279*, 35518–35525.

Beaulieu, J.M., Sotnikova, T.D., Marion, S., Lefkowitz, R.J., Gainetdinov, R.R., and Caron,

M.G. (2005). Cell *122*, 261–273.

Chao, J., and Nestler, E.J. (2004). Annu. Rev. Med. *55*, 113–132.

Chen, W., Ren, X.R., Nelson, C.D., Barak, L.S., Chen, J.K., Beachy, P.A., de Sauvage, F., and Lefkowitz, R.J. (2004). Science *306*, 2257–2260.

Ferguson, S.S., Downey, W.E., 3rd, Colapietro, A.M., Barak, L.S., Menard, L., and Caron, M.G. (1996). Science *271*, 363–366.

Kang, J., Shi, Y., Xiang, B., Qu, B., Su, W., Zhu, M., Zhang, M., Bao, G., Wang, F., Zhang, X., et al. (2005). Cell, this issue.

Lohse, M.J., Benovic, J.L., Codina, J., Caron, M.G., and Lefkowitz, R.J. (1990). Science *248*, 1547–1550.

Luttrell, L.M., Ferguson, S.S., Daaka, Y., Miller, W.E., Maudsley, S., Della Rocca, G.J., Lin, F., Kawakatsu, H., Owada, K., Luttrell, D.K., et al. (1999). Science *283*, 655–661.

Scott, M.G., Le Rouzic, E., Perianin, A., Pierotti, V., Enslen, H., Benichou, S., Marullo, S., and Benmerah, A. (2002). J. Biol. Chem. *277*, 37693–37701.

Shenoy, S.K., and Lefkowitz, R.J. (2003). Biochem. J. *375*, 503–515.

Wang, P., Wu, Y., Ge, X., Ma, L., and Pei, G. (2003). J. Biol. Chem. *278*, 11648–11653.

Wilbanks, A.M., Fralish, G.B., Kirby, M.L., Barak, L.S., Li, Y.X., and Caron, M.G. (2004). Science *306*, 2264–2267.

The Battlefield of Pluripotency

Austin Smith[1,2,*]
[1]Centre Development in Stem Cell Biology, Institute for Stem Cell Research, School of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JQ, United Kingdom
[2]Institute for Stem Cell Biology, University of Cambridge, Tennis Court Road, Cambridge CB2 1TN, United Kingdom
*Contact: austin.smith@ed.ac.uk
DOI: 10.1016/j.cell.2005.11.012

How embryonic stem cells maintain the potential to differentiate into multiple cell lineages is still unclear. In this issue of *Cell*, Niwa et al. (2005) show that a duel between the transcription factors Oct3/4 and Cdx2 can restrict embryonic stem cells to either embryonic or placental fate. The vulnerability of lineage potential to transcriptional perturbation may reflect an essential feature of pluripotency.

Pluripotency is the capacity of a cell to generate all lineages of the developing mammalian embryo, including the germline. This is the unique and essential property of the epiblast, a population of founder cells located within the developing blastocyst. These epiblast cells form in conjunction with the segregation of extraembryonic and embryonic lineages during the preimplantation phase of development (Figure 1). Epiblast cells multiply transiently until gastrulation, during which they then develop into either primordial germ cells or somatic progenitor cells. In vitro, the progression to gastrulation can be interrupted and pluripotency sustained indefinitely by deriving self-renewing embryonic stem (ES) cells. How pluripotency is acquired by a subset of cells in the embryo and how it is maintained in ES cells are questions of intellectual fascination and increasingly of biomedical significance. A series of incisive experiments by Niwa and colleagues (2005) reported in this issue of *Cell* provide a conceptual framework for considering these phenomena.

Niwa et al. (2005) focus attention on Oct3/4, a master transcriptional organizer of the POU transcription factor family. Oct3/4 expression is found exclusively in early embryos, the germline, and ES cells. In the embryo, Oct3/4 is essential to establish pluripotency (Nichols et al., 1998). Without Oct3/4, inner cells of the blastocyst fail to acquire the potential to differentiate into multiple lineages and are restricted to the generation of a single lineage, the extraembryonic trophectoderm, which eventually gives rise to the placenta. Furthermore, continuous expression of Oct3/4 is required to sustain pluripotency. The elimination of Oct3/4 from ES cells precipitates unidirectional differentiation into trophectoderm (Niwa et al., 2000).

Oct3/4 is thus positioned foremost in the hierarchy of transcriptional determinants of the pluripotent state (Chambers and Smith, 2004). One key effect of Oct3/4 is to suppress differentiation of embryonic cells into trophectoderm. Niwa et al. (2005) demonstrate that Oct3/4 achieves this by blocking both the expression and activity of the homeodomain transcription factor Cdx2. First, they observed that Cdx2 is rapidly upregulated upon elimination of Oct3/4 from mouse ES cells. Then they found that forced expression of Cdx2 is sufficient to induce trophoblast differentiation (Figure 1, lower panel), reproducing the effect of removal of Oct3/4. The gene expression changes that accompany misexpression of Cdx2 or elimination